

AMENDMENTS TO THE SPECIFICATION

In the title:

IMMUNOLOGICAL TEST KIT COMPRISING AN WITH IMMUNOLOGICALLY INVISIBLE
PEG COPOLYMER CARRIER CONJUGATED TO ONE OR MORE IMMUNOLOGICALLY
REACTIVE SUBSTANCES

In the abstract:

Please delete the existing abstract and replace it with the following paragraph:

A novel compound comprising an immunologically invisible polyethylene glycol copolymer is used to carry one or more immunologically reactive substances. The novel compounds may be used as part of kits for immunological assays.

Please delete the paragraph beginning on page 1, line 5 and ending on page 1, line 13 and replace it with the following paragraph:

This application claims priority from Stanley STEIN et al., "Highly Sensitive and Specific IgM-Capture...", provisional patent filing serial no. 60/242,819, filed 24 Oct. 2000, which is ~~and Bo~~ ~~QUI et al., "Multiple Epitopes Connect By A Carrier," Serial No. 09/_____, filed Oct. 2001.~~ ~~The contents of these, together with Bo QUI, "Studies of Polymers" (unpublished) and Bo QUI et al., "Selection of Continuous Epitope Sequence," 55 Biopolymers 319 (2001) are incorporated~~ herein by reference.

Please delete the paragraph beginning on page 6, line 3 and ending on page 6, line 26 and replace it with the following paragraph:

Such polymers are known in the art. General reviews of such compounds include Langer, R., "Biomaterials in Drug Delivery," 33 Acc.Chem.Res. 94 (2000); and Langer, R., "Tissue Engineering," 1 Mol.Ther. 12 (2000). One example of such an immunologically invisible compound is a N-vinylpyrrolidone-methyl methacrylate co-polymer, perhaps with added polyamide-6. Buron, F. et al., "Biocompatible Osteoconductive Polymer, 16 Clin.Mater. 217 (1994). Another example is poly (DL-lactide-co-glycolide) capsules. Isobe, M. et al., "Bone Morphogenic Protein Encapsulated with a Biodegradable and Biocompatible Polymer," 32 J.Biomed.Mater.Res. 433 (1996). Another example is a 70:30 ratio mixture of methylmethacrylate: 2-hydroxyethyl methacrylate. Bar, F.W, et al., "New Biocompatible Polymer Surface Coating," 52 J.Biomed.Mater.Res. 193 (2000). Another example is 2-methacryloyloxyethyl phosphorylcholine, perhaps with polyurethane. Iwasaki, Y. et al., "Semi-Interpenetrating Polymer Networks..." 52 J.Biomed.Mater.Res. 701 (2000). Polyvinyl pyrrolidone may also be used, as may polyethylene glycol and its derivatives. Other biocompatible polymers ~~polymenrs~~ are known in the art. E.g., Haisch, A. et al., "Tissue Engineering of Human Cartilage Tissue," 44 HNO 624 (1996); Ershov, I.A., et al., "Polymer Biocompatible X-Ray Contact Hydrogel," 2 Med.Tekh. 37 (1994); Polous, I.M. et al., "Use of A Biocompatible Antimicrobial Polymer Film," 134 Vestn.Khir.Im.II Grek. 55 (1985).

Please delete the paragraph beginning on page 7, line 32 and ending on page 8, line 4 and replace it with the following paragraph:

We made a new polyethylene glycol with multiple functional groups and a favorable geometric arrangement to achieve strong and stable antigen-antibody binding ~~binding~~ for the selected epitope peptides. We used α , ω -diamino-polyethylene glycol to copolymerize with amino group-protected aspartic acid to obtain a new polyethylene glycol-aspartic acid copolymer. Multiple attachment sites become available for conjugation through the pendant amino groups of the aspartic acid residue upon removal of the protection (Figure 1).

Please delete the paragraph beginning on page 8, line 14 and ending on page 8, line 27 and replace it with the following paragraph:

The conjugation of epitope peptides may use thiol-specific chemistry under mild conditions. The easiest strategy for peptide conjugation is to add extra amino acid on either the amino ~~amino~~ or carboxyl terminus of the peptide to allow one-site coupling to the carrier. In our study design, a cysteine residue, followed by two β -alanine residues, was incorporated at the C-terminus of each epitope peptide during solid phase peptide synthesis. Putting two more β -alanine residues between the conjugation anchor, cysteine, and the epitope peptide is used as a precaution to generate further flexibility of the linear peptides, and therefore help them to adopt the optimal conformations for stronger antibody binding. The N-terminus of the peptides needs to be capped in order to remove charges associated with free amino groups and thereby mimics ~~mimicking~~ the real environment in the protein.

Please insert the following new paragraphs on page 10, between lines 15 and 16:

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1 shows the synthesis of a polyethylene glycol copolymer comprising multiple amino groups for peptide attachment.

FIGURE 2 shows the synthesis of a novel PEG-peptide conjugate comprising both peptides and reporter molecules.

FIGURE 3 depicts one point of attachment for reporter molecules on the aspartic acid and PEG conjugate, which is reporter attachment to the epitope peptide.

FIGURE 4 is a drawing of IgM-capture ELISA using PEG- peptide conjugate as antigen for serological diagnosis of Lyme disease.

Please delete the paragraph beginning on page 11, line 36 and ending on page 12, line 12 and replace it with the following paragraph:

For the final cycle, piperidine treatment was carried out right after the double coupling of active esters and DMF washing. Bromophenol blue solution was then added to obtain blue color for all spots and finally the peptides on each spot ~~were~~ was capped by acetylation. After synthesis and acetylation, the protecting groups present on the side chains of the amino acids must be removed ~~removed~~. For side chain deprotection, 5 mL of the DCM was mixed with 5 mL TFA. The mixed solution was added immediately onto the air-dried membrane and the cleavage reaction was allowed to proceed for 1 hour. The membrane was then washed with 3 x 20 mL DMF, and 3 x 20 mL methanol. The membrane was air-dried and stored in a sealed plastic bag in the freezer (-20 °C) until required for SPOTS analysis.